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13. ABSTRACT (Maximum 200 Words)

The combined case-comparison study and randomized controlled trial (RCT) of 90 women is based on our prior edpidemiologic work (1-6) indicating that vegetables in the *Brassica* genus (e.g., broccoli, cauliflower, brussel sprouts) can modify estrogen metabolism by causing 17β- estradiol (E2) to be metabolized to 2-hydroxyestrone (2HE) rather than 16α-hydroxyestrone (16HE) thus producing a cascade of effects protective against breast cancer (2). Our plan is to enroll 45 postmenopausal women with breast cancer and 45 age-matched disease-free women and to compare them on: 1) AhR activation and its various protein products relevant to cancer including CYP1B1, PAI-2, and IL-1; and 2) levels of relevant estrogens, E2, 2HE, and 16HE.

The RCT will examine the effect of an intensive *Brassica*-rich diet intervention on AhR activation, its protein products, and estrogen metabolites in these women. This study is completing its third year of (no-cost) activity. All protocols for the collection of data are finalized and we have recruited 83 participants. The baseline data have been collected for case-comparison study and the last intervention cycle for the RCT will be in September 2003. Specific accomplishments are described in the following narrative, in parallel with the original Statement of Work.

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7. Int J Epidemiol 1989; 18:518-526.

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Army Award DAMD17-99-1-9279

Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention

Annual Report: Year 4

This study is beginning its third full year of funded activity. We have finished recruiting participants using approved protocols for the collection of data. The first of four intervention cycles began in 2002. Specific accomplishments are described in the following narrative, in parallel with the original Statement of Work. We have enrolled a total of 83 women in the study (August 2003).

Introduction

Work by our group and others provide the scientific basis of this study (1-11). Cross-national studies of breast cancer rates and studies of migrants indicate that environmental factors are responsible for large population-level differences in breast cancer rates and rates of change over time. In a study of 46 countries, we found that over 90% of breast cancer mortality could be accounted for mainly by dietary factors (12). On a per-calorie basis, the strongest effect in the data was the protective effect of cabbage. There is some evidence that vegetables in the *Brassica* genus, like cabbage and broccoli, modify estrogen metabolism by causing 17β- Estradiol (E2) to be metabolized to 2-hydroxyestrone (2HE) rather than 16α-hydroxyestrone (16HE). Relative to 2HE, 16HE appears more likely to cause cancer and breast cancer patients have a lower ratio of these metabolites than do disease-free controls. It has further been shown that the P450 enzyme CYP1B1 is present in tumor but not normal breast tissue. The indole glucosinolates (IGSL), which are contained in high concentrations in *Brassica* vegetables, induce a number of protein products that can shift E2 metabolism away from 16HE and towards 2HE. AhR activation also induces immune system factors such as interleukin-1β (IL-1β) and other proteins, such as plasminogen activator inhibitor-2 (PAI-2), a protease inhibitor that has been associated with inhibition of tumor invasiveness (metastasis).

Specific Aims

The two objectives of this proposal are to evaluate the products of AhR activation against the risk of breast cancer, and to investigate the ability of *Brassica* vegetables to reduce breast cancer risk. Women will be recruited from among those who have undergone a diagnostic biopsy at SCCC following a suspicious mammogram. The plan is to enroll 45 postmenopausal women who have had breast cancer and 45 age-matched women found to be disease free. The first study, conducted at the time the women enter the study, will compare the 45 breast cancer patients and the 45 high-risk healthy women on: 1) AhR activation and its various protein products relevant to cancer including CYP1B1, PAI-2, and IL-1□; and 2) levels of relevant estrogens, E2, 2HE, and 16HE. The second study will examine the effect of an intensive *Brassica*-rich diet intervention on AhR activation, its protein products, and estrogen metabolites in these 90 women. Measurement of all study parameters will be made at times corresponding to

the baseline period and post-intervention. Blood and fasting morning urine samples will be collected for measurement of the estrogens, and levels of PAI-2 and IL-1□. Adipose tissue for assay of CYP1B1 will be collected from routine open biopsy at the time of recruitment and from a fine needle biopsy of the contralateral breast at follow-up. Diet will be assessed by use of validated diet assessment instruments. Compliance also will be assessed by levels of isothiocyanates and dithiocarbamates in urines. Statistical analyses of the data will consist of t-tests and analysis of variance of mean levels of the parameters specified in the three groups at baseline. T-tests of change and regression analyses (e.g., repeat measures ANOVA) will focus both change and relative change in the intervention trial. Post hoc analyses will examine the effect of the indole carbinols by fitting the data as continuous, which takes into account varying levels of compliance.

Distinctive subject terms

- *Brassica* vegetables- vegetables belonging to the Brassica genus including cabbage, broccoli, cauliflower, spinach, collards, and Brussels sprouts
- *Brassica* diet- consuming an intensive Brassica-rich diet
- Indole glucosinates (IGSL)- Dietary indoles are contained in Brassica vegetables and converted in the body to aryl hydrocarbon receptor (AhR) agonists that bind to AhR and induce CYP1 enzymes.
- Aryl hydrocarbon receptor (AhR)- has a role in inducing protein products that can shift E2 metabolism away from 16HE and towards 2HE. It has a role in inducing immune system factors (e.g., interleukin-1β and other proteins (e.g., plasminogen activator inhibitor-2)
- Hydroxyestrone- two forms of this hormone are created using the 17β-Estradiol precursor (e2) including 2-Hydroxyestrone (2HE, less toxic form) and 16α-Hydroxyestrone(16HE, more toxic form)
- Cytochrome P1B1 (CYP1B1)- a phase I enzyme present in tumor but not in breast tissue

The primary hypotheses are:

1. Examine if there are differences in AhR and its protein products, including CYP1B1, PAI-2, and IL-1β and estrogen metabolites at baseline in two subsets of women who have undergone diagnostic open breast biopsy at SCCC;
2. If intensive Brassica vegetable intake can alter levels of these products and estrogen metabolites through intensive dietary intervention on Brassica vegetable intake; and
3. If there is a relationship between CYP1B1 and estrogen metabolites, both cross-sectionally and longitudinally.

Work Accomplished

In previous years we modified all questionnaires being used and obtained access to the Palmetto Health's (PH) Cancer Data Management System tumor registry database. We have completed active recruitment of potential study participants. Potential women have been identified from the PH's Tumor Registry, through Breast Cancer Support Groups, local press releases and from Breast Care Centers at both Richland and Baptist hospitals. Intervention cycles will end in September 2003.

Task 1: Run-in Phase, Months 1-12:

a. Inventory and finalize all assessment instruments and data collection protocols.

Assessment instruments have been inventoried and are available for use. Final versions of all assessment instruments have been produced, as stipulated in the protocol. Copies of these instruments are included in the appendix.

Below is a list of instruments being utilized.

Baseline questionnaire Measures include: Background and Demographic Data: age; sex; marital status; education; number of children; number and dates of pregnancies; breast feeding history: (months for each child); and menopausal status (including surgical menopause). Personal Health History: present medical/psychiatric history and treatment (including history of exposure to estrogens, oral contraceptives, unusual menstrual problems). Family Health History: history of breast cancer; history of other cancers. General Self Care: sleep; exercise frequency; and smoking status.

Besides data collected on the baseline instrument we will also administer these other questionnaires:

- Marlowe-Crowe Social Desirability (MCSD) scale (Personal Reaction Inventory)
- Social Approval Scale
- Multiple 24-Hour Recall Phone Interviews [note that we have changed to this method as it appears to ease participant burden and is associated with lower overall measurement error (13).]
- Vegetable and Fruit Questionnaire [the paper validating this was published recently (14)
- Monitoring questionnaire
- Intervention Course Book, which includes intervention descriptions, food preparation methods, a cook book, telephone numbers of study personnel, and a brief description of the purposes of the study

New data collection protocols have been developed to fully utilize all resources under development at USC. As part of standard recruitment procedures, we mail an introductory letter and consent form to potential participants. We follow-up this letter with a telephone calls, and answer any questions regarding the study. As part of recruitment, a meeting is scheduled at the study center located within the South Carolina Cancer Center (SCCC). The SCCC facility includes an interview room, sample processing lab, and calibrated scales and measurement instruments. At the meeting, participants have the opportunity to ask additional questions regarding the consent form. After obtaining consent, we obtain a urine sample, blood sample, buccal cells, body size measurements, and participants complete the baseline questionnaire. Follow-up measurements are collected using a similar mechanism. Additionally, near the end of the intervention a clinic appointment is scheduled for collection of breast biopsy material, blood sample, a fasting urine sample, body size measurements, and participants complete a vegetable and food survey (see above).

b. Review baseline questionnaires for completeness and for content validity.

All instrument materials have been thoroughly reviewed and validated.

c. Revise baseline questionnaire to assess demographic, health history, and family health history, as necessary.

The Baseline Questionnaire has been expanded to include a more complete description of each participant's health history and demographic status. This expansion followed the move to USC, and the greater population diversity in SC as compared to Massachusetts. The questionnaire has been pilot tested, and appears to be sufficiently clear and complete.

d. Hire and train the Research Assistant.

Several personnel have been hired in order to complete this, and other, research projects. Dr. James Hebert has been the Principal Investigator for the project throughout, and Dr. Jay Fowke has assumed the role of consultant. Dr. Stephanie Muga remains the as Co-Investigator for the study. Wendy McKenzie continues as Project Coordinator. Mary Modayil, a USC doctoral student in the Department of Epidemiology and Biostatistics will be largely responsible for the day-to-day operations of the project. Thomas Hurley functions as a full-time data manager. His primary responsibility focused on developing the tracking databases necessary for ensuring complete recruitment and data collection. Additionally, he is responsible for questionnaire maintenance, questionnaire development, and data entry. Denise Crawford and Jennifer Heinz are students at USC. Zhihong Gong is a USC doctoral student in the Department of Epidemiology and Biostatistics. Their primary responsibility will be to assist Dr. James Hebert in contacting potentially eligible participants, mailings, and data management.

e. Develop the study data management systems, using a combination of Lotus Notes, Microsoft Excel, and EpiInfo.

As mentioned in the previous report, we have developed an improved data management system using optical scanning technology and the Teleform software package. Lotus Notes was not used in this study as we have moved to more universally recognized solutions. All questionnaires are now optically scanned, thus avoiding operator error associated with keypunching data, and greatly speeding the data entry process. Optically scanned data are directly transferred to a SAS dataset for analysis, thus eliminating most of the need for EpiInfo.

f. Develop the tracking database in Microsoft Access and Microsoft Excel based on our experience with other intervention studies in the Department of Epidemiology and Biostatistics.

We have completed an extensive database system, which links directly with the clinical hospital patient bases and other ongoing cancer studies. This data management system is

able to rapidly identify potentially eligible women receiving care at one of the cancer centers. This information is converted to the study-specific tracking system, used for maintaining records of recruitment, participant status, and data collection.

g. Train staff in all data-related and clinic-based procedures.

We have trained staff to conduct all data-related procedures. Dr. Hebert, Mr. Hurley, and Ms. Modayil are responsible for the overall data management and statistical analysis. Mr. Hurley, the data manager, has received formal training in the Teleform software package and extensive experience using the SAS software package. The graduate research assistants have been trained in the application of Teleform and they are developing the skills necessary to perform many routine SAS data management operations. They also have been trained to collect body size measurements using standard and systematic protocols, as well as in urine collection, sample preparation, and storage protocols. The biopsy collection protocol will be conducted by one of the members of the Radiology Department with the PH hospital network.

h. Develop and finalize all laboratory procedures to be used in the trial.

The majority of laboratory procedures will be conducted by Dr. Dawen Xie at USC. With the exception of the CYP1B1 assay, all necessary laboratory protocols are commercially available as kits. Members of Dr. Xie's lab have extensive experience in forming radioimmunoassays and enzyme immunoassays as required through use of these kits.

i. Finalize all biological sample collection and storage procedures to be used in the study.

All biological sample collection and storage procedures for urine and blood are finalized. The biopsy collection protocol has been developed in order to maximize volume of epithelial cells from breast tissue, due to new published findings suggesting better methods to detect CYP1B1 in breast tissue. The assay protocol is almost finalized with the help of Dr. Xie's lab with the goal of increasing sensitivity of the antibody to the CYP1B1.

j. Establish recruitment procedures for women entering the study, including pre-screen for certain criteria such as menopausal status.

Recruitment procedures were established, and recruitment is completed. We are mailing an initial recruitment card to heighten awareness of the study. This is being followed by an information letter relating more study details. Both of these recruitment methods mentioned the phone interview during which we collected pre-screening information on personal characteristics, diet, medication use, and health history. We have developed the data management system such that we will be able to identify women who receive a negative screening (healthy) and women who eventually are diagnosed with breast cancer.

k. Finalize the intervention protocol.

We have finalized the intervention protocol, based on our experiences with past dietary interventions. An intervention syllabus has been generated, listing specific content and topics for each class. Our dieticians, Brook Harmon, Lori Myers, Anna Dynarski, and Corinne Cates will lead weekly group discussions on incorporating Brassica vegetables into a daily diet, menu planning, and preparing quick healthy meals. Intervention materials have been generated, including a course booklet, 3-day diet diaries, a brief vegetable questionnaire, a brief monitoring questionnaire designed to measure adverse reactions or changes in health-related behaviors, and a recipe book. Dietary goals have been set. Rapid conversion of self-reported compliance levels will allow participants to monitor compliance relative to peers. We have identified several dieticians in Columbia who are sufficiently skilled to lead the intervention, and we are confident in our ability to hire such an intervention leader at the appropriate time.

Task 2: Recruitment, Months 12-24:

a. Identify women who could be eligible for the study from among those visiting the Breast Clinic at Palmetto Richland Hospital for the purpose of an open biopsy as a part of a diagnostic work up following a suspicious mammogram. We also identified former breast cancer cases from the PH Tumor Registry Database who may be eligible to take part in this study.
b. We implemented procedures for recruitment through the PH clinical services. We were able to identify women receiving breast biopsy procedures and who were eligible for the study among those visiting the PH participating hospitals. Recruitment began in January 2002).
c. Among those who stated they were willing to participate, we determined eligibility using the 18 criteria listed in section 4.1 of the proposal. We have developed a simple eligibility screening form suitable for use in the large-scale screening of potential participants during a telephone interview.
d. Abstract medical records for relevant health history and pathology data. The PH Tumor Registry contains information on pathology and the history of the first course of treatment for women with a previous diagnosis of the disease. For women currently visiting the Breast Clinic at Palmetto Health Richland and Baptist Hospital campuses, we are able to link their medical records with eligibility criteria in order to enroll them into this study.
e. Randomize to either intervention or control. Inform woman of this. When woman attend their 1st clinic visit, they are assigned to the intervention or non-intervention group.
f. Enroll the consecutive eligible women who have histologically confirmed stage I or II cancer of the breast.
g. Enroll consecutive eligible women who are disease free and meet all eligibility requirements of the study and are matched to the cases on age (±5 years). We completed data collection on 82 women who are disease free and met all eligibility requirements of the study.
h. Schedule the first clinic appointment for the purposes of collecting all of the blood and urine specimens and taking the anthropometric measurements.
i. Ensure that the open biopsy material is processed and sent to Dr. Xie's laboratory. Biopsy material is kept frozen and in storage.

j. Collect data on lifestyle, demographic, and health (family and personal history) plus psychosocial factors as outlined in 4.4.3. We use the baseline survey for this.
k. The dietician contacts each participant randomized to the intervention and schedules the group sessions. If the participant cannot attend all classes, the dietician conducts individual sessions with the participant on the telephone.

Task 3: Intervention / Passive Follow Up in the Controls, Months 14-28 (all items subsumed here are completed):

Ensure that the intervention is delivered according to the protocol.

a. Through collaboration with a local cardiac rehabilitation center, we arranged access to an appropriate conference room and adjoining teaching kitchen.
b. Encouraged women randomized to the intervention to attend all of the sessions. The dietician contact women to encourage them to attend. The women are provided adequate vegetables for the intervention during the group sessions.
c. As proposed, we stayed in contact with the control group to assure compliance with the follow-up measures.
d. Follow-up visits were conducted at the Breast Clinic for the blood, urine, and anthropometric data collection.
e. At this visit the fine-needle aspirate (FNA) was collected.
f. Assure that all self-assessments were completed at follow up.

Task 4: Data Entry, Verification and Interim Analyses, Months 12-28 (all items subsumed here are on-going):

a. All data were successfully read into the tracking and analytic databases.
b. All outlier and illogical responses were flagged and verified.
c. We have been conducting simple descriptive analyses (e.g., cross-tabulations and univariate statistics) since year 2.

Task 5: Final Data Analyses, months 28-36 (these tasks are on-going):

a. Perform all exploratory analyses to test for adherence to model assumptions.
b. Perform all necessary data manipulations (e.g., log transforming all non-normal and heteroschedastic data).
c. Test study hypotheses.
d. Conduct post-hoc analyses of study data.
e. Prepare manuscripts.
f. Archive datasets for future analyses and future patient follow-up.
g. Plan for future studies.

Key Research Accomplishments are all subsumed under the Task List, as noted above.

Reportable Outcomes, in addition to those things noted above, include two papers of relevance to this study including one on using isothiocyanate excretion as a biological marker of *Brassica* vegetable consumption (14) and the other on nutrient intake and estrogen metabolism in healthy postmenopausal women (15). Copies of these are included in the appendix. We also have produced a large number of measurement instruments that are included in the Appendix as well. Preliminary data analysis was presented at the Era of Hope Conference in September 2002. (see appendix)

Conclusion: After experiencing delays with study start up due to issues around Human Use, this study is now on track in terms of research deliverables.

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Appendices

Appendix 1: Consent

Approved Institutional Review Board Consent Form

On file:
Baseline Questionnaire
Vegetable and Fruit Questionnaire
Side-Effects and Reactions Form
Recruitment Card
Letter of Introduction
Phone Script
Screening Survey
Urine
Blood
Body Size Measurements
Draft Syllabus
Food Lists and Dietary Goals
24-HR Recall Script
Era of Hope Poster Presentation

Palmetto Health Alliance

INFORMED CONSENT FOR PARTICIPATION IN RESEARCH

IRB#: 2000-78

TITLE: Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention.

PRINCIPAL INVESTIGATOR: James R. Hebert, Sc.D.

RESEARCH SUBJECT'S NAME: _____ **DATE:**_____

SPONSOR: United States Department of Defense

INVITATION TO TAKE PART AND INTRODUCTION:

You are invited to volunteer for a research study. You have been asked to be in this study because you have undergone a screening procedure at the South Carolina Cancer Center within Palmetto Health Alliance (Columbia, S.C.) to see if you might have breast cancer.

PURPOSE OF THE RESEARCH:

The main purpose of this study is to determine if a 6-session dietary education program can help women incorporate into their diet certain foods that could alter levels of hormones thought to influence the risk of breast cancer. These foods are members of the *Brassica* genus. The most commonly consumed of these vegetables include cabbage, broccoli, cauliflower, and Brussels sprouts. The results of this study will help to develop dietary guidelines directed towards breast cancer prevention and altering the course of disease in women with breast cancer.

YOUR RIGHTS: It is important for you to know that:

- **YOUR PARTICIPATION IS ENTIRELY VOLUNTARY.**
- **YOU MAY DECIDE NOT TO TAKE PART OR DECIDE TO QUIT THE STUDY AT ANY TIME.**
- **YOU WILL BE TOLD ABOUT ANY NEW INFORMATION OR CHANGES IN THE STUDY THAT MIGHT AFFECT YOUR PARTICIPATION.**
- **THE QUALITY OF CARE YOU RECEIVE AT THE HEALTH CENTER WILL NOT BE AFFECTED IN ANY WAY IF YOU DECIDE NOT TO PARTICIPATE OR IF YOU WITHDRAW FROM THE STUDY.**

IRB APPROVAL

07 11 2003 - 07 01 2004

Subject's Initials _____ Witness's Initials_____

RANDOMIZATION:

Because it is not known whether changes in diet are effective in breast cancer prevention, not everyone in the study will be assigned to receive the dietary intervention. You will be assigned to one of two groups. One group will receive the dietary intervention, one group will not. This will make it possible for us to judge the effect of eating these vegetables in the fairest, most impartial way possible because the process of randomization ensures that the two groups of people (those receiving and those not receiving the intervention) are similar in other ways. The decision as to whether you receive the dietary intervention or not will be made by chance, like the flip of a coin, not by your doctor or based on your medical condition. You will have a 50% chance of receiving the intervention.

PROCEDURES:

This dietary study will last about two months per participant, and 90 women will participate. You will be asked to participate in a total of 12 different 24-hour dietary recalls during the study period (before, during, and after). If you are assigned to the dietary intervention, you will be asked to meet with a study dietitian for a one-hour individual session. This session will be followed by 6 two-hour group sessions over a three or four week period. Approximately fifteen people will attend each class, and classes will be scheduled on one or two weekday evenings. These sessions will include: 1. classroom presentations during which we will provide information about the vegetables – their chemical properties and their effects on health; 2. a group cooking experience in which you will be asked to learn about preparing the foods; and 3. a chance to eat what you have cooked with other women in the group.

You will be asked to add about four commonly known vegetables to your diet during the six weeks of the intervention. We will <u>not</u> be asking you to restrict your diet, or limit the other foods that you eat, in any way. The dietary intervention is not a weight loss program. You may eat anything that you wish to eat, but we ask that you also eat about two or three servings per day of the vegetables promoted in the intervention classes. These classes are designed to help you incorporate these vegetables into your normal meals.

We will ask to schedule two clinic visits with you. The first clinic visit will be scheduled at a time before the intervention starts, and the second visit will occur near the end of the intervention. During each of these clinic visits, a blood sample will be drawn in the usual way, by inserting a needle into a vein in your arm. About 4 teaspoons (20 milliliters) of blood will be collected, and this blood will be used to determine if there are any changes in levels of the hormones that are thought to be important in modifying breast cancer risk. We will measure your weight and the circumference of your hips and waist. We will provide you with a small urine collection container to collect a first-morning urine sample, and this urine sample can be brought to the clinic when you have your blood drawn. It is important that this urine sample be collected before you eat that day. This urine sample will be used to determine if there are changes in the levels of certain female hormones (estrogens) that are excreted from your body in your urine. Additionally, urine samples will be used to determine the levels of chemicals that naturally exist in the foods you will be asked to eat. We also will collect a small number of breast cells. This will be done by a procedure called a fine-needle aspiration, using a needle similar to that used for drawing blood. The amount of material removed by a fine-needle aspiration is always very small, less than a one-quarter of a thimble-full. This material will be used to determine levels of enzymes that are important in regulating levels of female hormones (estrogens).

IRB APPROVAL

07 11 2003 - 07 01 2004

Subject's Initials _____ Witness's Initials_____

In summary, each of the two clinic visits will include:

- Collection of a blood sample
- Delivery of a first-morning urine sample
- Collection of a small amount of breast material by fine-needle aspiration
- Measurement of your weight, waist, and hips

Finally, you will be asked to complete several questionnaires about your present health, diet, medication use, and the current level of depression and anxiety. These questionnaires will be completed near the time of your clinic visit, and will require about one hour.

After the end of the week of your last class, you will be advised that you may remove the intervention vegetables from your diet.

ALTERNATIVES:

You may choose not to take part in this study. If so, then you would not have to do any of the things listed above. This would in no way affect other aspects of your treatment or medical care.

RISKS AND INCONVENIENCES:

Drawing blood may hurt slightly, and you might have a bruise. Occasionally a person may become dizzy or faint when blood is drawn and there is a slight possibility of infection or temporary nerve damage. There may be pain associated with the fine-needle aspiration. This pain is usually short-lived (i.e., less than 12 hours), and well tolerated. Pain medication, for example Tylenol or Advil, can be taken to relieve this pain, and Tylenol capsules will be available at the time of the biopsy. Stronger pain medication may be prescribed if you think it is needed. There may be a small amount of bleeding which would present no health risk. There is a slight possibility of infection. Sterile techniques are used to avoid infection, but antibiotics can be used to treat an infection if this occurs. There is a very slight risk of temporary nerve damage, which should begin to heal within a few days. There should be no risk from answering any of the study questions, or in providing a urine sample.

Sometimes people find a question on a questionnaire sensitive or uncomfortable to answer. While there are reasons why the question is asked, you do not have to answer a particular question if you feel uncomfortable to do so. Please remember, all results will remain confidential. When we do the statistical analyses for the entire study we will not reveal your identity or the identity of anyone else in the study.

Adverse or allergic reactions to the foods promoted by the dietary intervention are rare. Occasionally, individuals have reported that consumption of the intervention foods leads to excess gas or diarrhea. We will ensure that you are in weekly contact with the project nutritionist and other research staff, and we will encourage you to call if you suspect any side effects. If any side effects occur, you may be advised to eat fewer of the vegetables.

Incorporation of a few additional foods to the diet may at times be an inconvenience when dining out or visiting people. There also may be inconvenience when planning or preparing meals for others in your home. The intervention class content and project staff will try to provide as much help as reasonably

Subject's Initials_____ Witness's Initials_____

possible to overcome such inconveniences and to make these changes enjoyable. Through discussion and conversation, other classmates also may be able to help with these issues.

COMPENSATION IN CASE OF INJURY:

All forms of medical diagnosis, treatment and research, whether routine or experimental, involve some risk of injury. In spite of all precautions, you might develop complications from participation in this study. In the event of any injury resulting directly from the research procedures, neither the study personnel, the University of South Carolina, nor Palmetto Health Alliance have made any provision for the payment of any financial compensation to you or to provide any financial assistance for medical or other costs.

This study is being funded by the Department of Defense and conducted by the United States Army in conjunction with the University of South Carolina. Army regulations provide that, as a volunteer in a study conducted by the United States Army, you are authorized all necessary medical care for any injury or disease that is a direct result of your participation in the research. The Principal Investigator or his designee will assist you in obtaining appropriate medical treatment under this provision, if it is required. If you have any questions concerning your eligibility for Army-funded medical treatment you should discuss this issue thoroughly with the Principal Investigator or his designee before you enroll in this study. This is not a waiver or release of your legal rights.

BENEFITS:

This study may be of no direct benefit to you. However, we will make study results available to you when the final results are compiled and written. At the end of the study, you may request a summary of all of your own results with a brief description of what they mean. As results from the entire study are published, we will advise you and you may request them as well. Additionally, the knowledge gained from your participation in this research may help to better understand how to prevent or treat breast cancer.

COSTS:

There will be no direct cost to you for participating in the study. The analyses of questionnaires, blood, urine, aspiration material, and the dietary intervention classes will be provided free of charge.

If you are assigned to the dietary intervention, you will receive a supply of vegetables during each class that can be incorporated into the regular diet. This is done as a convenience to you, and the amount of vegetables supplied should be more than enough to meet the intervention objectives. However, such supplies are intended to be eaten by the study participant, and there will not be a sufficient quantity to share with others. In the event that you wish to share the provided vegetables with friends or family members, we would ask that you purchase additional vegetables.

REMOVAL FROM STUDY

You may be taken out of the research study if it appears that you are unable to: keep your appointments, provide blood, urine, two fine-needle aspiration samples, or do not provide answers on the questionnaires. If this occurs, you will be given a full explanation.

IRB APPROVAL

07/14/2003-07/01/2004

Subject's Initials_____ Witness's Initials_____

CONFIDENTIALITY:

Your research records will be confidential. In all records of the study you will be identified by a code number and your name will be known only to the researchers. Your name will not be used in any reports or publications of this study.

Because this study is funded by the United States Department of Defense it has a special set of requirements known as "Volunteer Registry Data Base Requirements". It is the policy of the U.S. Army Medical Research and Materiel Command (USAMRMC), the entity that regulates this research, that data sheets are to be completed on all volunteers participating in research for entry into this Command's Volunteer Registry Data Base. The information to be entered into this confidential database includes your name, address, Social Security number, study name and dates. The intent of the database is two-fold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that the USAMRMC can exercise its obligation to ensure research volunteers are adequately warned (duty to warn) of risks and to provide new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command are eligible to review research records as a part of their responsibility to protect human subjects in research.

SAMPLE DONATION:

During this study, you will be asked to provide two breast fine-needle aspiration samples, two blood samples, and two urine samples. These samples will be used for hormone analysis related to breast cancer research. They also may be used for purposes that are currently unknown. There is a chance that the samples that you are donating under this study may be used in other research studies and may have some commercial value. No commercial value is anticipated at this point. Should your donated sample(s) lead to the development of a commercial product, the University of South Carolina will own it and may take action to patent and license the product. The University of South Carolina does not intend to provide you with any compensation for your participation in this study nor for any future value that the sample you have given may be found to have. You will not receive any notice of future uses of your sample(s).

PATIENT PROTECTION:

Further information on the research to be performed, or on any risks, benefits or alternative treatments may be obtained from James R. Hebert at 803-777-7666. This study has been approved by the committee to protect human rights for Palmetto Health Alliance. Information concerning your rights as a research subject can be obtained by contacting the Office of Corporate Counsel at (803) 296-2124.

IRB APPROVAL

07112003-07012004

Subject's Initials _____ Witness's Initials_____

Consent to Participate in the research project **IRB #2000-78**, entitled:

Phase I Induction and Estrogen Metabolism in Women With and Without Breast Cancer and in Response to a Dietary Intervention

Subject's name: (printed or typewritten)_____
P.I. Name: James R. Hebert, Sc.D.

"The purpose and procedures of this research project and the predictable discomfort, risks, and benefits that might result have been explained to me. I have been told that unforeseen events may occur. I have had an opportunity to discuss this with the investigator and all of my questions have been answered. I agree to participate as a volunteer in this research project. I understand that I may end my participation at any time. I understand that there is a possibility that the blood, tissue, or urine samples, which I am providing under this study, may also be used in other research studies and could potentially have some commercial applicability. I have been given a copy of this consent form."

Qualified Person Obtaining Consent: _____

Subject's signature:_____ **Date:**_____

Subject's permanent address: _____

_____

Witness signature: _____ **Date:**_____

_____ _____
Witness' name (printed or typewritten) **Relationship to subject**

IRB APPROVAL

07112003-07012004

Subject's Initials _____ Witness's Initials_____